2018-02

Hidden in plain sight: species richness and habitat characterisation of sublittoral pebble beds

Evans, J

10.1007/s00227-018-3292-4
Marine Biology
Springer Verlag

Hidden in plain sight: species richness and habitat characterisation of sublittoral pebble beds

Julian Evans[1,2]*, Martin J. Attrill[2], Joseph A. Borg[1], Peter A. Cotton[2] and Patrick J. Schembri[1]

[1]*Department of Biology, University of Malta, Msida MSD2080, Malta*

[2]*Marine Biology and Ecology Research Centre, Plymouth University, Plymouth PL4 8AA, UK*

* Corresponding author:

Email: julian.evans@um.edu.mt

Tel: (+356)99801241

ORCID ID: 0000-0001-7837-5927

Abstract

Sublittoral pebble beds are generally considered to be impoverished, but the physical and biological characteristics of these habitats are poorly known. We characterised nineteen pebble bed sites in the Maltese Islands, providing detailed habitat data for Mediterranean sublittoral pebble beds for the first time. Nearly 40,000 individuals belonging to 332 taxa were recorded in all, with total richness estimated to reach 440 taxa; molluscs, crustaceans and polychaetes were the most diverse faunal groups. This high diversity is likely due to the structural complexity of the pebble beds, which had a vertically stratified arrangement of sediment particles that is likely maintained through periodic physical disturbance. Variation in the biotic assemblage from site to site was correlated with changes in the quantity of sand

and silt, with the area of the pebble bed, with water depth, and with the thickness of the pebble layer. This indicates that pebble-bed macrofaunal assemblages are sensitive to changes in hydrodynamic conditions and sediment loading, to alterations to the stratification of the pebble beds, and to fragmentation of the habitat patches. These results contradict assertions that sublittoral pebble beds are impoverished, instead showing that they can be highly diverse habitats supporting biotic assemblages that respond to a complex set of environmental variables. The present findings enable better understanding of the ecological importance of pebble beds and of the potential impacts that may be caused by anthropogenic disturbance, thereby enabling more informed decisions for habitat conservation and management.

Keywords: Biodiversity, Conservation, Cobble bed, Community composition, Habitat, Malta, Mediterranean

Introduction

The 1992 Rio de Janeiro Convention on Biological Diversity heralded the "era of biodiversity" (Boero 2010) where exploration of diversity patterns and their environmental correlates became more imperative than ever. Nonetheless, to date the spatial patterns of marine benthic diversity remain poorly known for many habitats (Costello et al. 2010). Sublittoral cobble and pebble beds are a case in point. Quantitative descriptions of cobble or pebble biota are rare, possibly because conventional sampling methods such as cores or grabs are not suitable for these habitats (Linnane et al. 2003). We are aware of only five studies published in the mainstream literature that looked at the entire macrofaunal assemblage of

these habitats, based on sites in Canada, England, Ireland, France (Atlantic coast) and Norway (Scheibling and Raymond 1990; George et al. 1995; Linnane et al. 2003; Ringvold et al. 2015; Foveau and Dauvin in press). The few other studies that have been undertaken on these habitats focused exclusively on specific groups such as macrophytes (Lieberman et al. 1979, 1984; Davis and Wilce 1987a, 1987b; Scheibling et al. 2009a, 2009b), sessile biota (Osman 1977; Balazy & Kuklinski 2003, 2007), or decapods (Robinson and Tully 2000a, 2000b; Linnane et al. 2001). Of these, only Robinson and Tully (2000a) investigated the spatial variability in community structure in relation to substratum characteristics, but they also included areas that consisted predominantly of coarse sand or bedrock amongst their five sites.

Consequently not much is known about the physical or biological characteristics of sublittoral pebble and cobble habitats. These habitats are generally considered to be faunally impoverished (Connor et al. 2004; Foveau and Dauvin in press), a view possibly extrapolated from intertidal cobble and pebble habitats which have been described as "intertidal deserts" where macrobiota are absent (Little 2000). Accordingly, habitat classification systems used for marine conservation and management assume that there is little of interest in ecosystems comprising pebbles and cobbles. Yet this view does not appear to have been borne out by empirical studies, since the limited information available indicates that these habitats may not be so depauperate. For instance, between 15 and 32 decapod species were recorded from each of six shallow-water cobble sites in Norway, England, Ireland and Italy (Robinson and Tully 2000b; Linnane et al. 2001; Ringvold et al. 2015), while the faunal descriptions of another three sites in England, France and Ireland included a total of 382, 140 and 132 taxa respectively (George et al. 1995; Linnane et al. 2003; Foveau and Dauvin in press).

A similar situation is found in the Mediterranean Sea, where pebble bed habitats are also considered to be faunally impoverished (Bellan-Santini et al. 1994, 2002) despite the paucity of biological information on these assemblages (Bellan-Santini et al. 2002; Relini and Giaccone 2009). Given the presumption that cobble and pebble beds are depauperate, these habitats were not included in the reference list of Mediterranean marine habitats of conservation interest produced in connection with Barcelona Convention (UNEP-MAP-RAC/SPA 2006a). Yet in 2006, a population of the Maltese top-shell *Gibbula nivosa* was discovered from a pebble bed in Marsamxett, Malta (Evans et al. 2010, 2011). This is a critically endangered species endemic to Maltese waters, which has been afforded protection through the Bern Convention, the Barcelona Convention, and the European Union's 'Habitats Directive' (as an Annex II species) due to its risk of extinction (Evans et al. 2011). Subsequent work confirmed the importance of shallow-water cobbles and pebbles as a habitat for *G. nivosa* (Evans and Schembri 2014), indicating that pebble beds can be of conservation interest and highlighting the need for characterisation studies of these habitats.

Within this context, the present study was carried out to characterise the physical and biological features of sublittoral pebble beds in the Maltese Islands, thus providing detailed habitat data for Mediterranean sublittoral pebble beds for the first time, and enabling assessment of their species diversity. A second objective of this study was to identify correlations between the physical parameters and biotic components of the habitat. Recognition of such patterns is a necessary precursor for the formulation of hypotheses on the relationship between the physical nature of the pebble habitat and the species assemblage it supports (Underwood et al. 2000). Knowledge on the correlation between species assemblages and environmental characteristics is also vital for conservation efforts, since it

enables prediction of how these assemblages may change in response to environmental changes, including those associated with anthropogenic activities.

Methods

Study sites

A preliminary snorkelling survey was made along the low-lying coast of the Maltese Islands to map sublittoral pebble beds. Nineteen sites (Figure 1) were selected for further study, based on the following criteria: (i) the site consisted predominantly of pebbles (stones having a diameter of 2–10 cm following the UNEP-MAP-RAC/SPA [2006b] criteria), which lacked a covering of erect macrophytes; (ii) the site had an area of at least 25 m^2 of pebble habitat present at a depth of more than 0.5 m below chart datum. Areas with cobbles or small boulders that had a permanent cover of erect algae were excluded because they are considered to belong to a different assemblage – that of infralittoral algae (e.g.Bellan-Santini et al. 1994).

Sediment sampling and laboratory analysis

At each site, divers measured water depth using a digital depth gauge and estimated the dimensions of the pebble beds using fibreglass tape measures. Four replicate samples of the substratum were collected from each site; their position was selected at random, but no samples were collected within 2 m of the perimeter of the beds to avoid edge effects. A 0.1-m^2 circular sampler (modified from Borg et al. 2002) was pushed into the substratum to delineate the sampling area and prevent escape of motile invertebrates. Pebbles were then hand-collected and transferred to a 0.5-mm mesh bag, while a small hand net was used to

125 scoop the basal layer of finer granules into a separate 0.5-mm mesh bag. A suction sampler
126 was simultaneously employed to collect highly-motile organisms, thus ensuring quantitative
127 sampling of the whole assemblage. The thickness of the pebble layer was estimated by
128 measuring the thickness of the exposed section in comparison with the adjacent undisturbed
129 region. The 0.1-m^2 sampler allowed quantitative sampling of pebbles >8 mm, but not of finer
130 sediments. Therefore an intact core sample (area: 0.0016 m^2) of the granule layer and
131 underlying fine sediments up to a depth of 5 cm was also collected for grain-size analysis. All
132 fieldwork was carried out between July and September 2011.
133
134 Samples for biotic analysis were preserved in 10% formaldehyde in seawater. They were
135 subsequently sorted for macrofauna (retained by a 0.5-mm sieve; e.g. Castelli et al. 2003),
136 retaining all pebbles >8 mm for granulometric analysis. The biota were identified to the
137 lowest taxon possible and enumerated to construct a taxon-abundance matrix and obtain
138 estimates of number of taxa and abundance per sample.
139
140 For particle-size analysis, all pebbles >8 mm (collected using the 0.1-m^2 sampler) were
141 manually sorted into size classes at half-phi intervals on the Udden-Wentworth scale using a
142 gravel sizing template (Hydro Scientific Ltd.). Particles <8 mm (collected using the 0.0016-
143 m^2 sampler) were treated with sodium hexametaphosphate, wet-sieved for the determination
144 of silt content, then dried and separated into size classes at phi-intervals using a series of
145 nested test-sieves (see Bale and Kenny 2005). Since the finer sediment was collected using
146 smaller corers, the masses of the particle-size fractions <8 mm were scaled up using the ratio
147 of the area of the two corers as the scaling factor. Grain-size data were analysed using the
148 method of moments to obtain the geometric mean particle size, using the GRADISTAT
149 program (Blott and Pye 2001). Since bimodal distributions were common, the mean particle

size for the gravel fraction (particles >2 mm) was calculated independently of any finer particles, which were then quantified separately in terms of the percentage sand and silt content.

Measurements of other sediment characteristics related to pebble shape and roughness were undertaken since these determine the way pebbles are 'packed' in a bed, and hence the shape and size of interstitial spaces (and therefore microhabitats) available. These measurements were made on subsamples consisting of ten pebbles randomly selected from each size fraction (>8 mm), for a total of 50 pebbles per subsample. Surface area was estimated from the maximum dimensions (*L*, *W*, *H*) of the particles according to the equation: *Surface Area* = 1.15*(*LW*+*LH*+*WH*) (see Graham et al. 1988). Stone resistance to rotation was calculated as the moment of inertia of an ellipsoid ($I = m*(b^2+c^2)/5$; where *m* is the mass, and *b* and *c* are the lengths of the two minor semi-axes). Particle shape was characterised by: (i) form, in terms of elongation (*W*/*L*) and flatness (*H*/*W*) (Blott and Pye 2008); (ii) roundness, using a visual comparator (Blott and Pye 2008); and (iii) sphericity, as the square-root of the ratio of the largest inscribed circle diameter to the smallest circumscribed circle diameter (Riley 1941), averaged across the three dimensions of the pebbles. Percentage cover of encrusting and filamentous algae was also recorded.

Physico-chemical characterisation of the water

Sampling was undertaken at monthly intervals over a period of one year (July 2011 – June 2012). Temperature, salinity, dissolved oxygen and turbidity were measured using an *in situ* meter (YSI 6820 multiparameter sonde) deployed just above the seafloor and two replicate seawater samples were collected from just above the pebble habitat using a Van Dorn water sampler. Nitrate and phosphate levels were determined using an automated continuous flow

analyser (Skalar SAN++ coupled with an SA1000 autosampler). Annual average concentrations for these parameters were calculated following standard practises in water quality monitoring (E.C. 2009). While physico-chemical parameters are not properties of the pebble beds *per se*, they were also taken into consideration since some of the study sites were within a harbour area (Sites 6–11; see Figure 1) and likely receive higher nutrient inputs and therefore have higher productivity, while Site 5 was situated close to a thermal effluent which could also affect its biota.

Statistical analyses

Univariate indices of diversity were calculated for each site, and for the pebble-bed assemblage as a whole (across all sites). These included: observed taxon richness (S_{obs}), estimated total richness (S_{est}), total abundance (*TA*), diversity (Hill diversity ^1D), evenness ($^1D/S_{obs}$) and relative evenness (Pielou *J'*). The *Chao1* estimator (Chao 1984) was used to estimate S_{est} (see Foggo et al. 2003), while choice of evenness measures was based on the rationale of Jost (2010). Since functionally redundant species tend to be taxonomically related (Clarke and Warwick 1998), indices that take species' relatedness into account provide a broader view of biodiversity that potentially also includes functional aspects (Somerfield et al. 2008). Thus, taxonomic diversity (Δ), taxonomic distinctness (Δ^*) (Warwick and Clarke 1995) and average variation in taxonomic distinctness (Λ^+) (Clarke and Warwick 2001) were also estimated.

Multivariate analyses were carried out using the PRIMER v6 software (Clarke and Gorley 2006) with PERMANOVA+ v1 add-on (Anderson et al. 2008). For biotic data, analyses were based on a Bray-Curtis similarity resemblance matrix calculated on square-root transformed taxon-abundance data. The taxa that were characteristic of the pebble-bed assemblage were

200 determined using SIMPER analysis (Clarke 1993), while nMDS ordination was performed to
201 visualise the community pattern. The BVStep routine was subsequently used to find the
202 smallest possible subset of taxa that together describe most of the among-site variation
203 observed in the full dataset, using the "peeling" procedure as described in Clarke and
204 Warwick (1998). The ordination pattern of sites based on physical attributes was visualised
205 through PCA. Correlations between the similarity matrices obtained using the physical data
206 (based on Euclidean distance) and biotic data (based on Bray-Curtis) were tested using a non-
207 parametric form of the Mantel test (RELATE).
208
209 Distance-based multiple regression and redundancy analyses (DistLM and db-RDA; McArdle
210 and Anderson 2001) were then used to identify the physical variables correlated with
211 variation in the biotic assemblage. The analyses were made using the entire faunal dataset,
212 and then repeated for sub-sets consisting of mobile and non-mobile fauna analysed
213 separately. Non-mobile fauna included taxa that are either sedentary or permanently attached;
214 parasitic taxa were omitted from these analyses. Although the DistLM/db-RDA routines do
215 not require the assumption of multivariate normality, predictor variables should not be
216 heavily-skewed or contain extreme outliers (Anderson et al. 2008). Therefore, prior to these
217 analyses, Shapiro-Wilk normality tests were run and, where necessary, Box-Cox normalising
218 transformations were applied (see Sokal and Rohlf 1995). Since abiotic variables were
219 measured on different measurement scales they were also standardised to unit variance to
220 bring them to a common scale (Legendre and Legendre 1998). DistLM was carried out using
221 the transformed environmental variables after checking for multi-collinearity (no variables
222 were removed since Pearson's r was <0.9 in all cases). Forward sequential fitting was used to
223 select those variables with the highest explanatory power according to the adjusted-R^2
224 selection criterion. A db-RDA plot was subsequently produced to allow visualization of the

sites' ordination according to the multivariate regression models previously generated via DistLM. An unconstrained ordination plot was produced via non-metric multidimensional scaling (nMDS) for comparison with the constrained db-RDA plot.

Results

Physical characterisation

A summary of the various physical parameters quantified for the pebble-bed sites is provided in Table 1. Seven sites occurred at a water depth <2.5 m, while the rest were found at a water depth of 5–12 m. Most of the pebble beds covered a relatively small area (25–500 m^2) although two extensive beds (>3000 m^2) were present in Marsamxett Harbour. In all cases, the pebble bed had a layered structure, with an upper stratum of pebbles and a lower stratum of granules and sand that became progressively finer with sediment depth; a basal silty layer was also present in the deeper sites (Figure 2). The upper pebbly stratum consisted of around two to three layers of pebbles with an overall thickness of ca. 5–10 cm. These pebbles mostly fell within the 16–32 mm or 32–64 mm size classes, although some variation in mean pebble size was evident between sites. The six sites located within Marsamxett Harbour had the highest proportion of sand and silt and the highest algal cover, while shallower sites had the lowest content of fine sediment and the lowest algal cover. Shallow sites also had the highest total surface area, reflecting the slightly thicker pebble beds present at these sites.

In terms of particle-shape characteristics, the pebbles from all sites classified as "slightly elongate", "slightly flat", "rounded" and "moderately spherical", according to Blott and Pye's (2008) classification systems. There was minimal variation in annual mean values for

salinity, dissolved oxygen, turbidity and phosphate content between sites. Similar temperature values were also recorded at the different sites except at site 5, where the presence of a power station thermal effluent close to the pebble bed resulted in temperature being around 3°C higher. Some variation in levels of nitrate were also noted, with shallow sites having the highest values overall, although all values were within the expected range for Maltese inshore waters (Axiak 2004).

Biotic characterisation

A total of 39,993 individuals belonging to 332 macrofaunal taxa were recorded. Of these, 34,035 individuals (313 taxa) were recorded from the upper pebble stratum while 5,958 individuals (187 taxa) were found in the underlying layer of granules and finer sediments. The most diverse groups were the Mollusca (134 taxa), Crustacea (93 taxa) and Polychaeta (77 taxa). Spirorbinae spp. accounted for 45% of all individuals and 88% of the polychaetes, with nearly half of the spirorbids being recorded from a single site (Marsaxlokk; Site 4 in Figure 1). Polychaeta was the most abundant faunal group overall, but non-spirorbid polychaetes comprised only 6% of the total fauna (Figure 3). Other faunal groups included the Echinodermata, Cnidaria, Sipuncula, Nemertea, Tunicata and Actinopterygii which together accounted for only 1.5% of the total abundance. A larger richness of mobile fauna (246 taxa) was recorded compared to non-mobile fauna (82 taxa), but the total abundances of the two functional groups were very similar (49% mobile; 51% non-mobile).

The gradient of a taxon accumulation plot for the sampled sites (Figure 4) decreased after the fifth site, but did not reach an asymptote, with *Chao1* estimating a total taxon richness of 440 taxa across all 19 sites. The actual number of taxa recorded per site ranged from 37 to 125, with an overall mean of 81 taxa. Sites located at depths <2.5 m had approximately half the

number of taxa (mean: 49 taxa) as those found in waters >5 m deep (mean: 96 taxa), but a higher abundance was recorded from the shallower sites. This was mainly due to high abundance values of one or two dominating taxa at the shallower sites. Evenness values were overall low (max $^{1}D/S_{obs}$ = 0.4). Values of taxonomic diversity and distinctness were generally similar for all sites, with the exception of taxonomic diversity recorded from site 4 which was much lower (Δ = 6.8) than that recorded elsewhere (Δ >40). The mean, minimum and maximum values of these univariate diversity indices recorded from the 19 sites are indicated in Table 2.

The taxa identified as main contributors to similarity between the 19 sites are shown in Table 3; these are considered to be characteristic of the studied pebble-bed assemblages and include representatives of all three major taxonomic groups (Crustacea, Mollusca, Polychaeta) and one Echinodermata. These taxa were all recorded from at least 13 of the 19 sites, but only *Chondrochelia savignyi* and *Xantho pilipes* were found at all sites. The smallest subset of taxa that still captured the sample relationships seen in the full community pattern (BVStep using a cut-off of Spearman's $\rho > 0.95$) consisted of the following 16 taxa: *Alvania mamillata*, *Amphipholis squamata*, *Ampithoe ramondi*, *Athanas nitescens*, *Bittium latreillii*, *Bittium reticulatum*, *Calcinus tubularis*, *Cerithium renovatum*, *Columbella rustica*, *Gibbula varia*, *Ischnochiton rissoi*, *Lysianassa costae*, *Maera grossimana*, *Melita hergensis*, *Microdeutopus* spp. and Spirorbinae spp. Several of these were in common with the list of taxa contributing to similarity between sites identified through SIMPER analysis. Excluding these 16 taxa and repeating the BVStep analysis did not yield another subset of taxa that could attain the Spearman's ρ >0.95 criterion, but resulted in a subset of 35 taxa that still closely matched the overall multivariate pattern (Spearman's $\rho = 0.94$). The next iteration yielded a subset of 39 taxa with Spearman's $\rho = 0.90$; subsequent iterations failed to identify

another subset that could adequately describe the overall multivariate pattern (Spearman's $\rho <$ 0.80).

Linking physical and biological patterns

The resemblance matrices produced based on environmental parameters and taxon-abundance data were significantly correlated (RELATE: Spearman's $\rho = 0.47$; $p = 0.002$) indicating that the measured physical variables may be important factors structuring the biotic assemblage. When considering predictor variables individually, the multivariate multiple regression made using the entire macrofaunal dataset indicated that the sand and silt content was the physical parameter that accounted for the highest variance in species data, followed by coverage of the pebble beds, percentage cover of algae on the pebbles, and depth of the beds (Table 4). When the environmental factors were added sequentially to build a parsimonious model correlated to the variation in biotic composition, the first chosen factor was the percentage sand and silt content. This was followed by patch area, depth and layer thickness, with the four factors explaining 50.6% of the variation in assemblage structure (Table 5). Almost identical results were obtained when mobile and non-mobile taxa were analysed separately, with the main difference being the inclusion of nitrate concentration in the parsimonious model for non-mobile taxa (Tables 4 and 5). The nMDS (unconstrained) and db-RDA (constrained) ordination plots of the sites based on the full biotic dataset are shown in Figure 5. Only the physical factors selected in the DistLM models were included in the db-RDA plot; the first two axes account for 80% of the fitted variation, and 40% of the total variation in assemblage structure.

Discussion

A total sampling area of 7.6 m^2 of pebble habitat yielded 332 different macrofaunal taxa and just under 40,000 individuals. These numbers contradict previous assertions that sublittoral pebble beds are species poor and depauperate habitats (e.g. Bellan-Santini et al. 1994). The percentage abundance values of faunal groups recorded from the Maltese pebble beds was comparable to those reported by Linnane et al. (2001) from cobble beds found at similar depths (7–17 m) in Norway, England and Italy, where crustaceans were the dominant motile fauna followed by molluscs and polychaetes; in contrast, molluscs were dominant at an Irish cobble site (Linnane et al. 2001, 2003). These authors provide estimates for the number of decapod species, ranging between 15–32 species (with sampling areas of 7–20 m^2), similar to the 17 decapod species recorded by Robinson and Tully (2000a) from a different location in Ireland (depth 6–12 m; sampled area 5 m^2), to the 23 species recorded by George et al. (1995) from Norfolk, England (depth 10–18 m; sampled area 7.3 m^2), and to the 28 decapod species recorded from Maltese pebble beds (present work). Notwithstanding the wide geographical separation of these locations, a number of decapod taxa including *Athanas nitescens*, *Pisidia* spp. and *Xantho pilipes*, were common to several sites, and are therefore seemingly associated with pebble/cobble habitats.

The species richness recorded in the present study is comparable to that found in other Mediterranean habitats which are considered to be highly diverse, such as seagrass meadows and maerl beds. For instance, 244 macroinvertebrate species were recorded from a maerl bed in Maltese waters (sampling area = 4.8 m^2; Sciberras et al. 2006), while a similar study on motile macroinvertebrates associated with *Posidonia oceanica* meadows recorded 315 species (sampling area = 3.7 m^2; Borg et al. 2010). Overall, it is clear that while pebble-bed habitats are generally held to be species poor, the few studies that have been made on these

habitats indicate that this is not the case; they are overlooked and under-studied habitats, but not necessarily impoverished ones. In addition, the number of species reported from cobble and pebble substrata is likely an underestimate of the total macrobenthic richness of these habitats, since none of the studies undertaken took habitat edges into account (George et al., 1995; Linnane et al. 2001, 2003; Robinson and Tully, 2000a; present study). Habitat edges may support a different suite of fauna, possibly having additional species beyond those occurring in the habitat centres. On the other hand, the edge species richness will also depend on the identity and richness of the adjacent habitats.

In the present study, species rich assemblages were recorded both at the level of individual sites and, when considering the entire habitat, across all sites. The high within-site diversity may be due to an intermediate disturbance regime (Osman 1977), or to the physically complex nature of the substratum (Robinson and Tully 2000a, 2000b) that incorporates interstitial spaces of various dimensions providing microhabitats and refugia from predators for animals of different sizes (Linnane et al. 2003; Liversage et al. 2017). Physical disturbance via wave action is probably the major cause of pebble overturn during winter months, but may play a minor role during prolonged periods of calm weather that occur in summer, especially in sheltered sites. Field observations made during the present sampling indicated that bioturbation, especially by the striped red mullet *Mullus surmuletus*, may be an important cause of disturbance in the deeper sites. The lack of erect algae on the surface of the pebbles, even during the calmer summer months, suggests that the pebbles are unstable and do overturn regularly.

Physical disturbance may itself increase the habitat complexity by leading to stratification (as observed in the present study) through vertical winnowing, which involves loss of fine

material to the subsurface by passing down through the interstitial cavities between the pebbles as these are disturbed by water motion or bioturbation (Parker and Klingeman 1982). Stratification may also be partly due to the selective fine particle entrainment, since this renders the surface layer coarser than the subsurface material. Such mechanisms enable the pebbles to persist in an unburied state (Parker and Klingeman 1982). Stratification can have important biological consequences since benthic community structure is often related to sediment textural characteristics (Anderson 2008), which could lead to different species groups being present in the different strata at a given site, thus increasing species diversity.

Most of the characteristic species of Maltese pebble beds were included in the smallest subset of taxa that still adequately described the among-site variation observed in the full dataset. This suggests that differences between the 19 study sites are partly driven by changes in abundance of these main taxa. Nonetheless, an element of structural redundancy (*sensu* Clarke and Warwick 1998) was also present, given that two other species subsets that included a total of 74 taxa between them also closely matched the overall multivariate pattern. In addition, several taxa were rare, with over half of the taxa being recorded from only one to three sites. Due to their rarity, such taxa may not have contributed substantially to the overall multivariate pattern but they are important contributors to the overall diversity of the pebble-bed assemblages.

The environmental parameters and taxon-abundance data were significantly correlated, indicating that at least some of the measured physical variables may be important factors structuring the biotic assemblage. One of the main factors which was highly correlated with biotic variation was the sand and silt content, probably because it is a good proxy for the hydrodynamic regime present at a site and may also influence food availability. Other

physical variables included in the parsimonious models were patch area, depth, pebble layer thickness. Depth can be a proxy for several other factors, including wave energy, light intensity and phytoplankton concentration, all of which may have an influence on benthic assemblages. The pebble layer thickness is a measure of habitat complexity. On its own, this factor explained less than 10% of the biotic variation but could explain almost 7% of the variation over and above that accounted for by the other parameters already in the model. The inclusion of coverage in the models is interesting given that the pebble patches are essentially habitat islands surrounded by other habitat types and, according to the classic theory of island biogeography, island area is one of the main factors influencing the occurrence and abundance of species in islands (MacArthur and Wilson 1967). This may be due to patch area *per se*, but can also result from its correlation with other factors that directly regulate the community structure (Ricklefs and Lovette 1999).

In conclusion, the present study shows that claims that sublittoral pebble beds are impoverished do not stand up to scrutiny, especially in the case of beds found deeper than a couple of metres. This misconception is likely due to the absence of detailed studies on these habitats Indeed, a recent study on mobile gravels and pebbles in north-western France (25–66 m depth) reached a similar conclusion that these habitats support a "surprisingly diversified macrofauna" (Foveau and Dauvin in press). The presence of unique or rare species within pebble beds would further enhance their conservation value, but this can only be ascertained through detailed comparative studies of the species assemblages found in different habitats, which was beyond the scope of the present work. On the other hand, pebbles beds may still represent unique assemblages with different functionality, even if none of their component species occur solely in pebble beds.

Our results indicate that sublittoral pebble beds can be highly diverse, with the biotic assemblage responding to a complex set of environmental variables. In such a situation, changes to the physical environment can lead to a significant impact on the assemblage structure and composition. In particular, the results of this study indicate that pebble-bed macrofaunal assemblages are sensitive to changes in hydrodynamic conditions and sediment loading, to alterations to the stratification of the pebble beds, and to fragmentation of the habitat patches. This information will enable better understanding of the ecological importance of pebble beds and of the potential impacts that may be caused by anthropogenic disturbance, thereby enabling more informed decisions for habitat conservation and management. In the case of the Maltese Islands, live populations of the critically endangered endemic top-shell *Gibbula nivosa* have only been recorded from cobble and pebble habitats since the rediscovery of this species in 2006 (Evans et al. 2010, 2011; Evans and Schembri 2014; this study). Since *G. nivosa* is included in Annex II of the European Union's 'Habitats Directive' (species whose conservation requires designation of protected areas), infralittoral pebble beds should actually be considered as priority habitats when formulating conservation management plans and designating protected areas in Maltese coastal waters.

Compliance with ethical standards

Funding: The present work was partly funded by the European Union – European Social Fund through a Strategic Educational Pathways Scholarship (Malta) grant awarded to JE.

Conflict of interest: The authors have no conflict of interest.

Ethical approval: This article does not contain any studies with animals performed by any of the authors.

Acknowledgement

We are grateful to two anonymous referees for their comments on a previous version of this paper.

References

Anderson MJ (2008) Animal-sediment relationships re-visited: Characterising species' distributions along an environmental gradient using canonical analysis and quantile regression splines. J Exp Mar Biol Ecol 366:16–27

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth

Axiak V (2004) National action plan for Malta for the reduction and elimination of land based pollution. National diagnostic analysis for Malta. Unpublished Report, Malta; document submitted to the United Nations Environment Programme, Coordinating Unit for the Mediterranean Action Plan, through the Environment Protection Directorate of the Malta Environment and Planning Authority

Balazy P, Kuklinski P (2013) Mobile hard substrata - An additional biodiversity source in a high latitude shallow subtidal system. Estuar Coast Shelf Sci 119:153–161

Balazy P, Kuklinski P (2017) Arctic field experiment shows differences in epifaunal assemblages between natural and artificial substrates of different heterogeneity and origin. J Exp Mar Biol Ecol 486:178–187

Bale AJ, Kenny AJ (2005) Sediment analysis and seabed characterisation. In: Eleftheriou A, McIntyre A (eds) Methods for the study of marine benthos (3rd ed). Blackwell Science Ltd., Oxford

474 Bellan-Santini D, Lacaze JC, Poizat C (1994) Les biocenoses marines et littorales de la
475 Méditerranée, synthèse, menaces et perspectives. Musée National d'Histoire Naturelle, Paris
476 Bellan-Santini D, Bellan G, Bitar G, Harmelin JG, Pergent G (2002) Handbook for
477 interpreting types of marine habitat for the selection of sites to be included in the national
478 inventories of natural sites of conservation interest. UNEP-MAP-RAC/SPA
479 Blott SJ, Pye K (2001) GRADISTAT: A grain size distribution and statistics package for the
480 analysis of unconsolidated sediments. Earth Surf Process Landf 26:1237–1248
481 Blott SJ, Pye K (2008) Particle shape: a review and new methods of characterisation and
482 classification. Sedimentology 55:31–63
483 Boero F (2010) The study of species in the era of biodiversity: a tale of stupidity. Diversity
484 2:115–126
485 Borg JA, Attrill MJ, Rowden AA, Schembri PJ, Jones MB (2002) A quantitative technique
486 for sampling motile macroinvertebrates in beds of the seagrass *Posidonia oceanica* (L.)
487 Delile. Sci Mar, 66:53–58
488 Borg JA, Rowden AA, Attrill MJ, Schembri PJ, Jones MB (2010) Spatial variation in the
489 composition of motile macroinvertebrate assemblages associated with two bed types of the
490 seagrass *Posidonia oceanica*. Mar Eco Prog Ser, 406:91–104
491 Castelli A, Lardicci C, Tagliapietra D (2003) Il Macrobenthos di fondo molle. In: Gambi MC,
492 Dappiano M (eds) Manuale di metodologie di campionamento e studio del benthos marino
493 Mediterraneo. Biol Mar Medit 10(suppl):109–144
494 Chao A (1984) Nonparametric estimation of the number of classes in a population. Scand J
495 Stat 11:265–270
496 Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure.
497 Aust J Ecol 18:117–143
498 Clarke KR, Gorley RN (2006) PRIMER v.6: User Manual/Tutorial. PRIMER-E, Plymouth

499 Clarke KR, Warwick RM (1998) Quantifying structural redundancy in ecological
500 communities. *Oecologia* 113:278–289
501 Clarke KR, Warwick RM (2001) A further biodiversity index applicable to species lists:
502 variation in taxonomic distinctness. Mar Ecol Prog Ser 216:265–278
503 Connor DW, Allen JH, Golding N, Howell KL, Lieberknecht LM, Northen KO, Reker JB
504 (2004) The Marine Habitat Classification for Britain and Ireland, Version 04.05. JNCC,
505 Peterborough
506 Costello MJ, Coll M, Danovaro R, Halpin P, Ojaveer H, Miloslavich P (2010) A census of
507 marine biodiversity knowledge, resources, and future challenges. PLoS ONE 5(8):e12110.
508 Davis AN, Wilce RT (1987a) Algal diversity in relation to physical disturbance: a mosaic of
509 successional stages in a subtidal cobble habitat. Mar Ecol Prog Ser 37:229–237
510 Davis AN, Wilce RT (1987b) Floristics, phenology, and ecology of the sublittoral marine
511 algae in an unstable cobble habitat (Plum Cove, Cape Ann, Massachusetts, USA). Phycologia
512 26:23–34
513 E.C. (2009) CIS Guidance document No. 19: Guidance on surface water chemical monitoring
514 under the water framework directive. Office for Official Publications of the European
515 Communities, Luxembourg
516 Evans J, Borg JA, Schembri PJ (2010) Rediscovery of live *Gibbula nivosa* (Gastropoda:
517 Trochidae). Rapp Comm Int Mer Medit 39:507
518 Evans J, Borg JA, Schembri PJ (2011) Distribution, habitat preferences and behaviour of the
519 critically endangered Maltese top-shell *Gibbula nivosa* (Gastropoda: Trochidae). Mar Biol
520 158:603–611
521 Evans J, Schembri PJ (2014) The resurrection of *Gibbula nivosa* (Gastropoda: Trochidae).
522 Rapp Comm Int Mer Medit 40 :653

523 Foggo A, Attrill MJ, Frost MT, Rowden AA (2003) Species richness estimators Estimating
524 marine species richness: an evaluation of six extrapolative techniques. Mar Ecol Prog Ser
525 248:15–26
526 Foveau A, Dauvin J-C (in press) Surprisingly diversified macrofauna in mobile gravels and
527 pebbles from high-energy hydrodynamic environment of the 'Raz Blanchard' (English
528 Channel). Reg Stud Mar Sci. doi:10.1016/j.rsma.2017.08.011
529 George JD, Chimonides PJ, Evans NJ, Muir AI (1995) Fluctuations in the macrobenthos of a
530 shallow water cobble habitat off North Norfolk, England. In: Eleftheriou E, Ansell AD,
531 Smith CJ (eds) Biology and ecology of shallow coastal waters. Proceedings of the 28th
532 European marine biology symposium. Olsen & Olsen, Fredensborg (Denmark)
533 Graham AA, McCaughan DJ, McKee FS (1988) Measurement of surface area of stones.
534 Hydrobiologia 157:85–87
535 Jost L (2010). The relation between evenness and diversity. Diversity 2:207–232
536 Legendre P, Legendre L (1998) Numerical Ecology (2nd ed). Elsevier, Amsterdam
537 Lieberman M, John DM, Lieberman D (1979) Ecology of subtidal algae on seasonally
538 devastated cobble substrates off Ghana. Ecology 60:1151–1161
539 Lieberman M, John DM, Lieberman D (1984) Factors influencing algal species assemblages
540 on reef and cobble substrata of Ghana. J Exp Mar Biol Ecol 75:129–143
541 Linnane A, Ball B, Mercer JP, Browne R, van der Meeren G, Ringvold H, Bannister C,
542 Mazzoni D, Munday B (2001) Searching for the early benthic phase (EBP) of the European
543 lobster: a trans-European study of cobble fauna. Hydrobiologia 465:63–72
544 Linnane A, Ball B, Munday B, Browne R, Mercer JP (2003) Faunal description of an Irish
545 cobble site using airlift suction sampling. Biol Environ 103B:41–48
546 Little C (2000) The Biology of Soft Shores and Estuaries. Oxford University Press, Oxford

MacArthur RH, Wilson EO (1967) The Theory of Island Biogeography. Princeton University Press, New Jersey

Liversage K, Cole V, Coleman R, McQuaid C (2017) Availability of microhabitats explains a widespread pattern and informs theory on ecological engineering of boulder reefs. J Exp Mar Biol Ecol 489:36–42

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297

Osman RW (1977) The establishment and development of a marine epifaunal community. Ecol Monogr 47(1):37–63

Parker G, Klingeman PC (1982) On why gravel bed streams are paved. Water Resour Res 18:1409–1423

Relini G, Giaccone G (eds) (2009) Priority habitats according to the SPA/BIO protocol (Barcelona Convention) present in Italy. Identification sheets. Biol Mar Medit 16(S1):1–367

Ricklefs RE, Lovette IJ (1999) The roles of island area per se and habitat diversity in the species-area relationships of four Lesser Antillean faunal groups. J Anim Ecol 68:1142–1160

Riley NA (1941) Projection sphericity. J Sediment Petrol 11:94–97

Ringvold H, Grytnes J-A, Van der Meeren GI (2015) Diver-operated suction sampling in Norwegian cobble grounds: technique and associated fauna. Crustaceana 88(2):184–202

Robinson M, Tully O (2000a) Spatial variability in decapod community structure and recruitment in sub-tidal habitats. Mar Ecol Prog Ser 194:133–141

Robinson M, Tully O (2000b) Seasonal variation in community structure and recruitment of benthic decapods in a sub-tidal cobble habitat. Mar Ecol Prog Ser 206:181–191

Scheibling RE, Raymond BG (1990) Community dynamics on a subtidal cobble bed following mass mortalities of sea urchins. Mar Ecol Prog Ser 63:127–145

571 Scheibling RE, Kelly NE, Raymond BG (2009a). Physical disturbance and community
572 organization on a subtidal cobble bed. J Exp Mar Biol Ecol 368:94–100
573 Scheibling RE, Kelly NE, Raymond BG (2009b) Herbivory and community organization on a
574 subtidal cobble bed. Mar Ecol Prog Ser 382:113–128
575 Sciberras M, Rizzo M, Mifsud JR, Camilleri K, Borg JA, Lanfranco E, Schembri PJ (2006)
576 Habitat structure and biological characteristics of a maerl bed off the northeastern coast of the
577 Maltese Islands (central Mediterranean). Mar Biodiv, 39:251–264
578 Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological
579 research (3rd ed). W.H. Freeman & Co., New York
580 Somerfield PJ, Clarke KR, Warwick RM, Dulvy NK (2008) Average functional distinctness
581 as a measure of the composition of assemblages. ICES J Mar Sci 65:1462–1468
582 Underwood AJ, Chapman MG, Connell SD (2000) Observations in ecology: you can't make
583 progress on processes without understanding the patterns. J Exp Mar Biol Ecol 250: 97–115
584 UNEP-MAP-RAC/SPA (2006a) Reference list of marine habitat types for the selection of
585 sites to be included in the national inventories of natural sites of conservation interest. UNEP-
586 MAP-RAC/SPA
587 UNEP-MAP-RAC/SPA (2006b) Classification of benthic marine habitat types for the
588 Mediterranean region. UNEP-MAP-RAC/SPA
589 Warwick RM, Clarke KR (1995) New 'biodiversity' measures reveal a decrease in taxonomic
590 distinctness with increasing stress. Mar Ecol Prog Ser 129:301–305
591

Tables

Table 1

Mean (± SD), minimum and maximum values of various physical parameters recorded from sublittoral pebble beds at 19 sites in the Maltese Islands.

	Mean ± SD	**Range**
Depth (m)	5.0 ± 3.4	0.7 – 12.0
Patch Area (m^2)	806 ± 1422	25 – 3900
Thickness (cm)	5.28 ± 1.65	2.0 – 8.8
Mean Pebble Size (mm)	30.7 ± 15.0	12.8 – 68.0
Sand & Silt content (%)	18.9 ± 12.8	0.0 – 40.8
Mean Moment of Inertia (g cm^2)	198 ± 419	6.8 – 1511
Total Surface Area (m^2)	0.54 ± 0.20	0.3 – 1.0
Mean Elongation	0.74 ± 0.02	0.7 – 0.8
Mean Flatness	0.66 ± 0.04	0.6 – 0.7
Mean Roundness	0.69 ± 0.05	0.6 – 0.7
Mean Sphericity	0.75 ± 0.01	0.7 – 0.8
Filamentous algal cover (%)	6.5 ± 4.3	0.0 – 13.9
Encrusting algal cover (%)	14.0 ± 19.7	0.0 – 55.2
Annual Mean Temperature (°C)	20.65 ± 0.76	19.9 – 23.6
Annual Mean Salinity (psu)	37.94 ± 0.13	37.6 – 38.1
Annual Mean Dissolved Oxygen (%)	107.2 ± 5.1	93.4 – 113.7
Annual Mean Turbidity (NTU)	0.52 ± 0.58	0.1 – 2.7
Annual Mean [Nitrate] (μg L^{-1})	78.4 ± 115	15.7 – 513.5
Annual Mean [Phosphate] (μg L^{-1})	2.6 ± 1.3	1.7 – 7.0

Table 2

Mean (± SD), minimum and maximum values for various univariate diversity indices recorded from sublittoral pebble beds at the 19 study sites.

	Mean ± SD	**Range**
Observed taxon richness - S_{obs}	81 ± 27	37 – 125
Estimated taxon richness - S_{est}	122 ± 37	59 – 185
Total abundance - *TA* (ind. 0.4 m^{-2})	2105 ± 1996	409 – 9279
Hill diversity - ^1D	13.7 ± 9.1	1.3 – 38.8
Evenness - $^1D/S_{obs}$	0.2 ± 0.1	0.0 – 0.4
Relative evenness - *J'*	0.5 ± 0.2	0.1 – 0.8
Taxonomic diversity - Δ	65.2 ± 17.6	6.8 – 85.2
Taxonomic distinctness - Δ^*	88.6 ± 4.7	79.7 – 98.4
Average variation in taxonomic distinctness - Λ^+	318.0 ± 40.7	261.6 – 421.0

606

607 **Table 3**

608 Output from SIMPER analysis based on square-root transformed abundance data, identifying
609 main taxa contributing to similarity in faunal assemblage present at the 19 study sites.

Taxonomic group	**Species**	**Mean Abundance (√ind. 0.4 m^{-2})**	**Frequency of Occurrence (%)**	**Mean Similarity**	**Contribution to Similarity (%)**
Polychaeta Sabellidae	Spirorbinae spp.	10.3	78.9	4.06	12.11
Crustacea Tanaidacea	*Chondrochelia savignyi*	5.5	100	3.11	9.27
Crustacea Amphipoda	*Gammarella fucicola*	3.0	94.7	1.39	4.14
Crustacea Decapoda	*Xantho pilipes*	1.7	100	1.04	3.11
Crustacea Amphipoda	*Ampithoe ramondi*	1.9	94.7	1.04	3.09
Mollusca Polyplacophora	*Ischnochiton rissoi*	2.1	84.2	0.99	2.95
Crustacea Amphipoda	*Microdeutopus* spp.	2.0	89.5	0.87	2.59
Crustacea Decapoda	*Athanas nitescens*	1.8	84.2	0.86	2.56
Crustacea Amphipoda	*Melita hergensis*	2.4	68.4	0.82	2.46
Polychaeta Nereididae	*Nereis rava*	1.4	84.2	0.74	2.19
Crustacea Amphipoda	*Maera grossimana*	2.0	68.4	0.73	2.18
Echinodermata Ophuiroidea	*Amphipholis squamata*	1.7	84.2	0.72	2.16
Mollusca Gastropoda	*Gibbula varia*	1.7	68.4	0.70	2.10

610

611

612 **Table 4**

613 Results of DistLM analysis identifying physical variables correlated to the variation in biotic composition, based on the adjusted-R^2 criterion for
614 each variable taken individually (ignoring other variables), using (a) the entire macrofaunal dataset, (b) mobile taxa only, (c) non-mobile taxa
615 only. Analyses were performed using Bray-Curtis similarity to measure variance in biotic data. % var: percentage of variance in species data
616 explained by that variable; p-values lower than 0.05 are indicated in bold.

617

Variable	(a) All Fauna			(b) Mobile Fauna			(c) Non-mobile Fauna		
	% var	Pseudo-F	P	% var	Pseudo-F	P	% var	Pseudo-F	P
Depth	16.3	3.302	**0.004**	16.8	3.435	**0.003**	12.5	2.429	**0.027**
Layer Thickness	9.0	1.674	0.071	9.0	1.689	0.055	8.4	1.550	0.117
Patch Area	23.3	5.167	**0.001**	22.0	4.806	**0.001**	24.3	5.467	**0.001**
Mean Pebble Size	7.4	1.360	0.160	8.1	1.501	0.105	4.7	0.840	0.537
Sand & Silt content	24.3	5.447	**0.001**	22.4	4.912	**0.001**	24.6	5.536	**0.001**
Mean Elongation	2.9	0.504	0.983	3.4	0.596	0.910	2.7	0.480	0.929
Mean Flatness	5.3	0.951	0.463	5.5	0.990	0.445	4.4	0.777	0.623
Mean Roundness	6.5	1.181	0.257	5.5	0.992	0.420	7.9	1.453	0.151
Mean Sphericity	6.2	1.125	0.276	7.1	1.303	0.189	4.6	0.814	0.587
Mean Moment of Inertia	10.6	2.017	**0.031**	10.9	2.083	**0.023**	8.7	1.615	0.122
Total Surface Area	11.1	2.131	**0.031**	11.7	2.252	**0.012**	8.9	1.662	0.096
Filamentous Algal cover	16.7	3.404	**0.001**	16.4	3.325	**0.001**	15.6	3.150	**0.006**
Encrusting Algal cover	19.0	3.989	**0.001**	17.7	3.650	**0.002**	21.5	4.661	**0.001**
Annual Mean Temperature	6.6	1.207	0.216	8.3	1.547	0.065	4.0	0.708	0.692
Annual Mean Salinity	4.5	0.801	0.656	5.0	0.904	0.547	3.3	0.574	0.857
Annual Mean Dissolved Oxygen	6.5	1.173	0.248	6.2	1.128	0.302	5.5	0.991	0.409
Annual Mean Turbidity	6.5	1.185	0.237	6.6	1.197	0.250	6.8	1.248	0.237
Annual Mean Nitrate concentration	12.4	2.402	**0.010**	10.0	1.887	**0.041**	15.9	3.211	**0.009**
Annual Mean Phosphate concentration	14.9	2.971	**0.004**	14.7	2.932	**0.002**	15.0	3.008	**0.006**

Table 5

Results of DistLM analysis identifying physical variables correlated to the variation in biotic composition, based on the adjusted-R^2 criterion with forward-selection of variables, where the amount explained by each variable added to the model is conditional on variables already in the model (i.e. those variables listed above it), using (a) the entire macrofaunal dataset, (b) mobile taxa only, (c) non-mobile taxa only. Only variables up to the first highly non-significant *p*-value (>0.09) are included in sequential fitting. Analyses were performed using Bray-Curtis similarity to measure variance in biotic data. % var: percentage of variance in species data explained by that variable; cum. %: cumulative percentage of variance explained; p-values lower than 0.05 are indicated in bold.

	(a) All Fauna			
Variable	**% var**	**cum %**	**Pseudo-F**	**P**
Sand & Silt content	24.3	24.3	5.447	**0.001**
Patch Area	9.6	33.8	2.312	**0.001**
Depth	7.2	41.0	1.826	**0.013**
Layer Thickness	6.3	47.2	1.665	**0.039**
Mean Pebble Size	5.3	52.6	1.452	0.093
	(b) Mobile Fauna			
Variable	**% var**	**cum %**	**Pseudo-F**	**P**
Sand & Silt content	22.4	22.4	4.912	**0.001**
Patch Area	9.6	32.0	2.262	**0.002**
Depth	8.3	40.3	2.077	**0.007**
Mean Pebble Size	6.0	46.3	1.565	0.054
Layer Thickness	5.3	51.6	1.421	0.128
	(c) Non-mobile Fauna			
Variable	**% var**	**cum %**	**Pseudo-F**	**P**
Sand & Silt content	24.6	24.6	5.536	**0.001**
Layer Thickness	11.1	35.7	2.775	**0.003**
Patch Area	9.3	45.0	2.524	**0.005**
Annual Mean Nitrate concentration	6.7	51.7	1.935	**0.043**
Annual Mean Phosphate concentration	4.4	56.1	1.302	0.208

Figures with legends

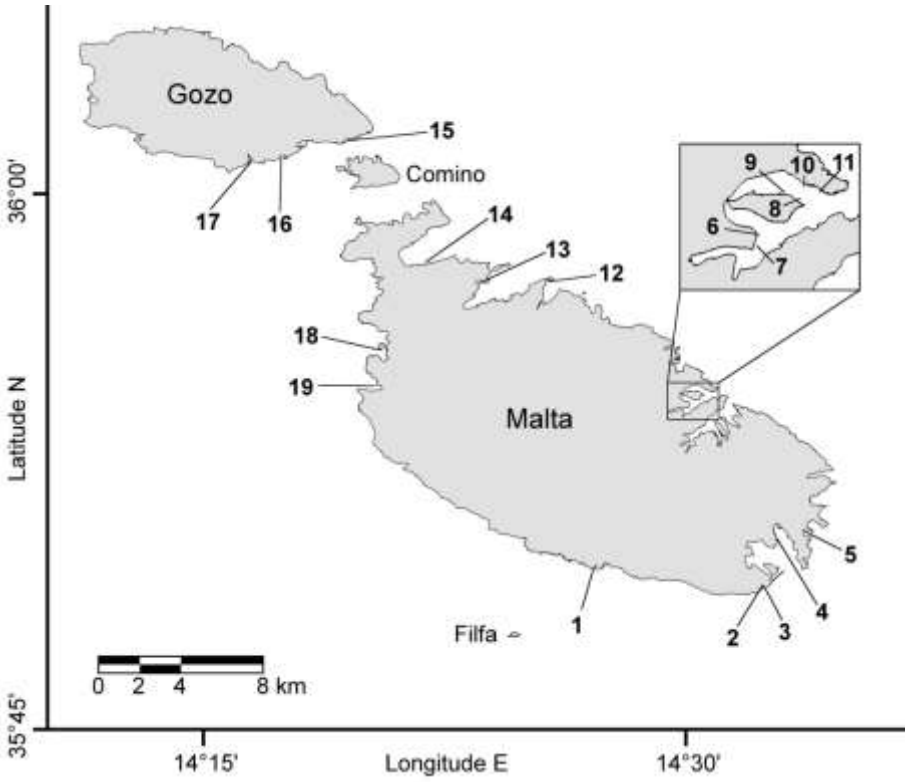

Figure 1 Map of the Maltese Islands indicating the location of the 19 sampling sites used in the present study. Inset shows enlarged view of Marsamxett Harbour.

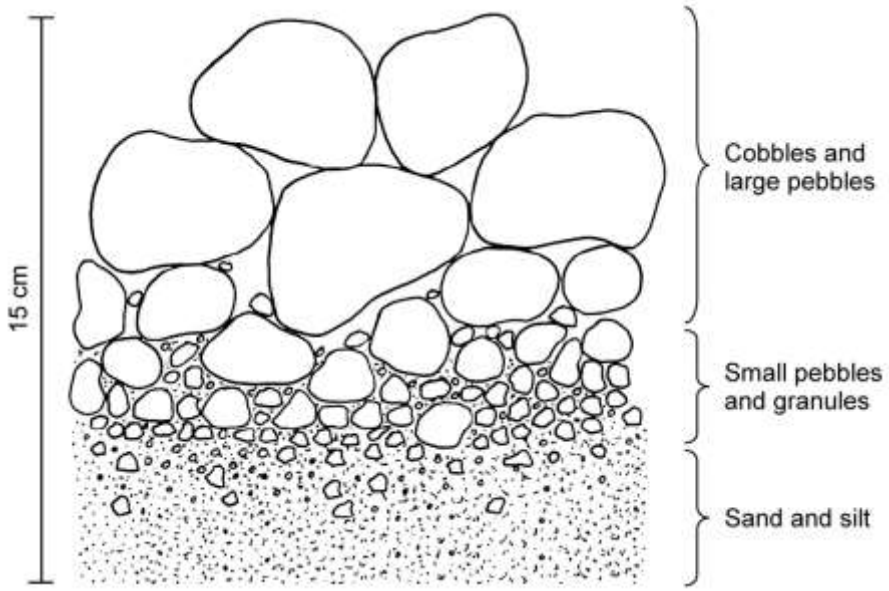

Figure 2 Diagrammatic pebble-bed profile showing vertical stratification, with an upper layer of pebbles and a lower layer of granules and sand; a basal layer of silty sand was typically present in harbour environments.

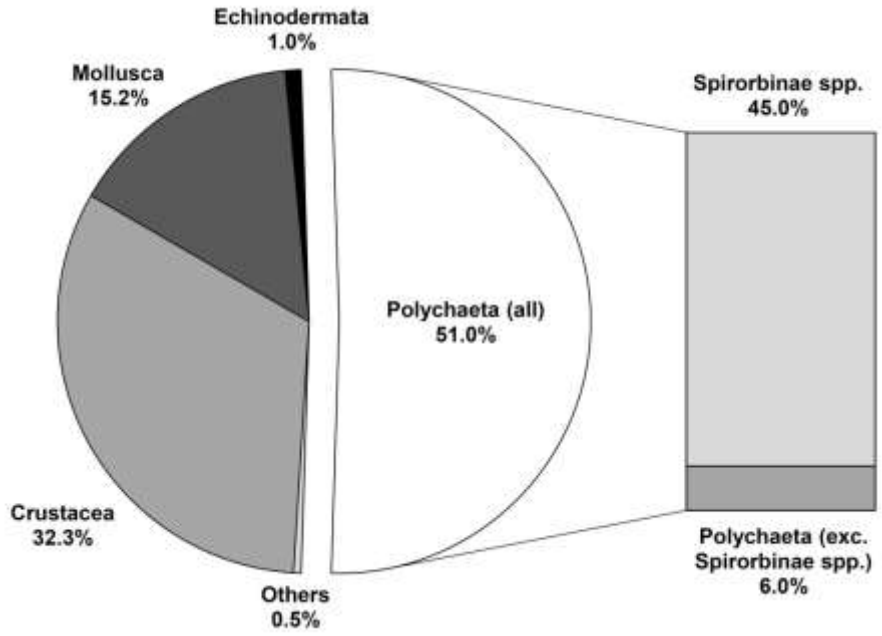

Figure 3 Percentage abundance of major faunal groups recorded from sublittoral pebble beds at the 19 study sites.

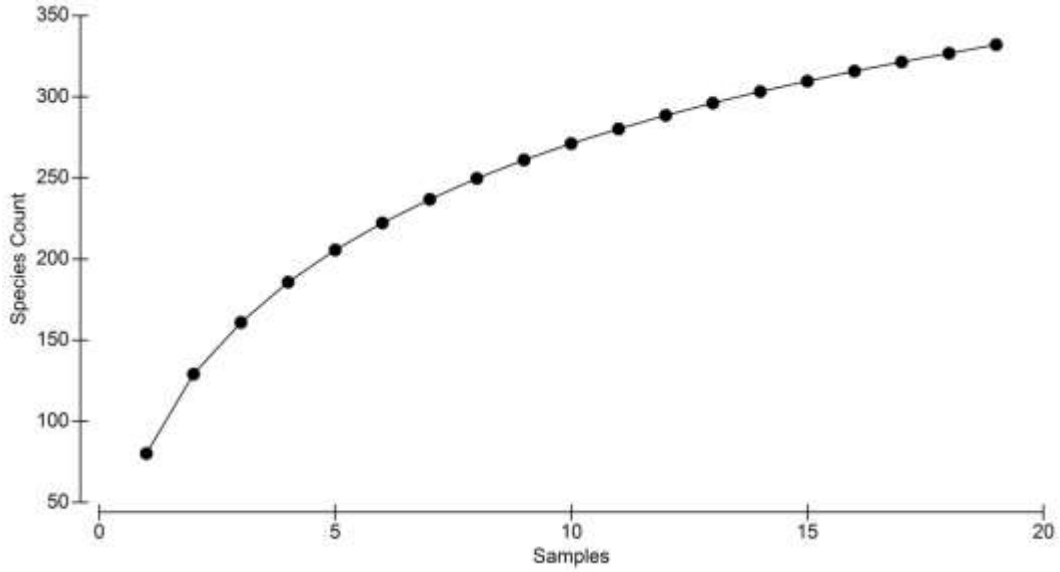

Figure 4 Taxon-accumulation plot showing cumulative number of taxa recorded from infralittoral pebble beds at the 19 study sites; the *Chao1* richness estimator indicated that the plot should reach an asymptote at a taxon richness of 440.

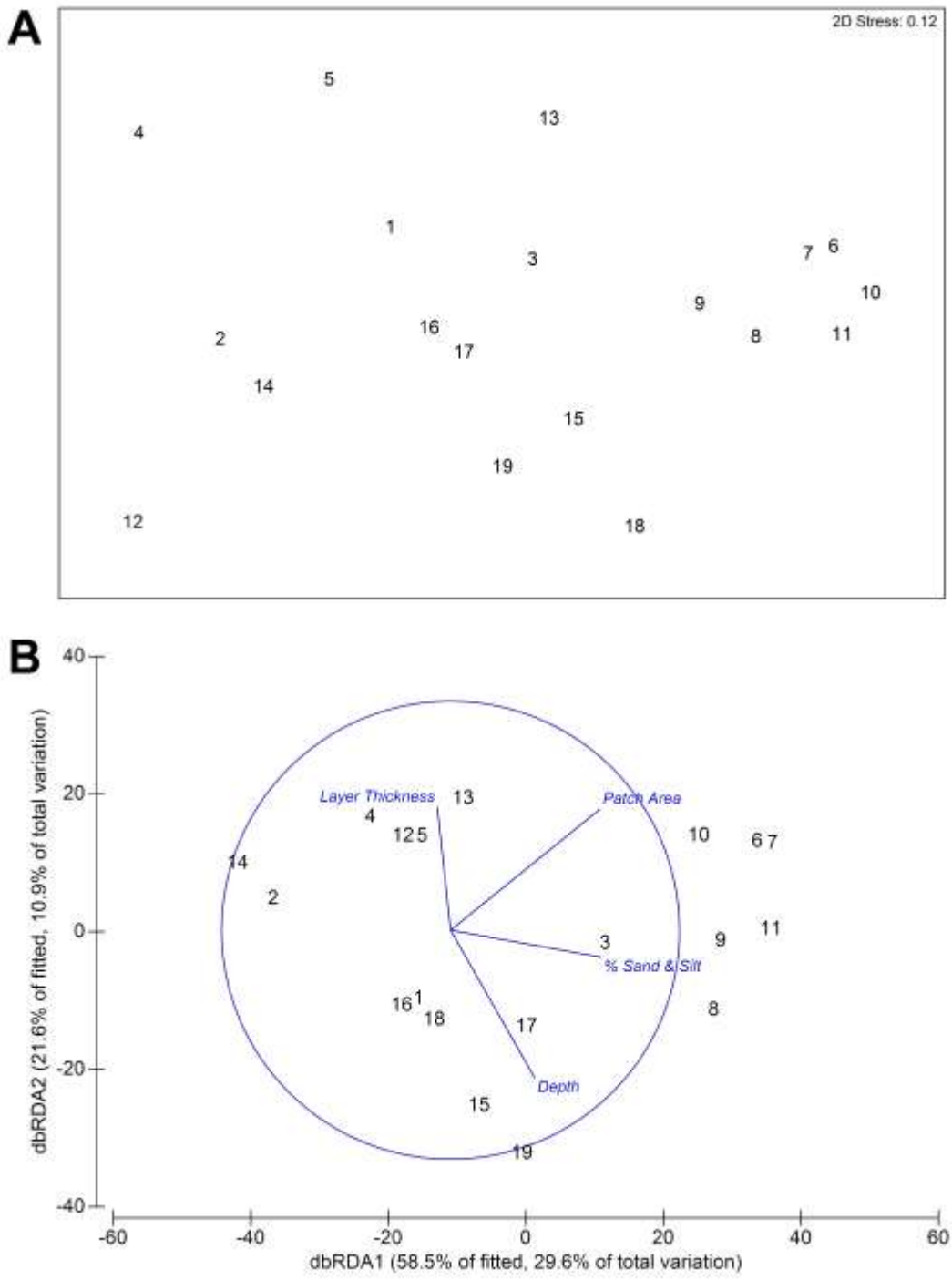

Figure 5 (a) Unconstrained non-metric multidimensional scaling ordination plot, where sites are positioned according to their similarity in biotic assemblage only. (b) Distance-based RDA ordination, where the ordination based on biotic data is constrained by their correlation with environmental variables, with vector projections of the physical factors selected by the DistLM routine; the length and direction of the vectors represent the strength and direction of the relationship between the variation in biotic assemblage and the physical variables. Both ordinations were performed using Bray-Curtis similarity of square-root transformed species counts.